ICTERUS.

A RAPID CHANGE OF HEMOGLOBIN TO BILE PIGMENT IN THE PLEURAL AND PERITONEAL CAVITIES.

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In an earlier communication (1) we have been able to show that bile pigment could be formed from hemoglobin without the agency of the liver. Solutions of hemoglobin were introduced into the blood vessels of dogs whose livers had been excluded from any part in this reaction. There was a prompt formation of bile pigment from hemoglobin with no possible direct liver action. This transformation can take place within a space of two hours when active circulation is maintained in the head and thorax alone. It seemed probable that the endothelium might be the tissue whose activity was responsible for this change of hemoglobin to bile pigments.

This work has received confirmation from experiments of McNee (2) who repeated the experiments of Minkowski and Naunyn with geese. He found that icterus did develop without liver activity and found evidences of endothelial activity. He suggests (3) that the Kupffer cells may normally transform hemoglobin to bile pigments.

The experiments given below prove conclusively that still other tissues can rapidly transform the hemoglobin pigment into bile pigment. There can be no question of any direct liver activity in these experiments. Some transformation may take place within eight hours, but the pigment production in twenty-four hours can usually be estimated with considerable accuracy. This mesothelium of the serous cavities can not effect this transformation as promptly as it is effected in the circulating blood, but the contact with the living pleural cells is not as active and intimate,—not a circulatory contact.
If we assume that the capillary endothelium can transform hemoglobin to bile pigment, it is at once obvious how intimate is the contact of the circulating hemoglobin solution with the living protoplasm of the vessels. The solution of hemoglobin in the serous cavities may be in more or less motion (respiration, peristalsis, or body movements), but at best it is not in very intimate contact with the living protoplasm. The wonder is that the transformation is so prompt and easily recognizable. It is impossible to estimate the relative activity of endothelium and mesothelium, but there can be little difference if we allow for the rapidity of circulation and surface contact.

It has been claimed by older workers (Virchow (4) and many others) that blood standing long in contact with living tissues was slowly changed to a golden pigment hematoidin which is chemically equivalent to bilirubin. Virchow used the term hemolytic icterus for this reason. Guillain and Troisier (5) noted in human cases with pleural hematomas that bile pigment was formed after a considerable period with no complicating liver abnormality. Von der Bergh and Snapper (6) were able to find greater amounts of bile pigment in serous exudates than in the blood serum, and they speak in favor of the extrahepatic formation of bile pigment. That the serous cavities can rapidly transform hemoglobin to bile pigment has not been hitherto recognized.

EXPERIMENTAL OBSERVATIONS.

The experiments are not all given in detail, but the essential facts are given in Tables I and II. A typical experiment from each group is given in sufficient detail, and it is to be emphasized that the majority of these experiments caused no inflammation or even permanent injury to the pleural or peritoneal cavity. In a few cases due to slips in technique there developed a pleurisy or a peritonitis, but these complications did not modify the transformation of hemoglobin to bile pigment. The crystalline hemoglobin causes a slight irritation in the pleural or peritoneal cavity, and it is possible that the inflammatory reaction may assist in the transformation, but it is not an essential factor.
Method.

Active and vigorous dogs were used in all of our experiments. The urine was obtained by catheter, and in every case it was examined before the experiment. The Huppert-Salkowski test for bile pigments was used in examining the urine, and unless otherwise stated 20 cc. of urine were employed in the test. Either fresh or crystalline dog hemoglobin was used. The fresh hemoglobin was obtained by laking washed erythrocytes with distilled water.

The preparation of crystalline hemoglobin was carried out according to the method of Bradley and Sansum (7). The red blood cells obtained from defibrinated dog blood by centrifugalization were washed eighteen or twenty times with normal salt solution, then they were laked with toluene and a little distilled water. The toluene layer was removed and the solution centrifugalized and decanted from the stroma material. This hemoglobin solution was then mixed with 20 per cent by volume of toluene, shaken thoroughly, and set aside in the cold. After twenty-four or forty-eight hours the mixture is nearly a solid mass of large well formed crystals. The crystals are collected by centrifugalization, washed with cold water, spread thin on glass, and dried in a stream of warmed air. Fresh 0.6 per cent salt solution was used as a diluting medium for the fresh or crystalline dog hemoglobin.

The fluids were introduced into or withdrawn from the pleural or peritoneal cavity through a trocar. After withdrawing the pleural or peritoneal fluids they were centrifugalized immediately, the supernatant fluid was decanted and made definitely alkaline by the addition of a saturated solution of sodium carbonate, then a 10 per cent solution of calcium chloride was added until precipitation was complete. After centrifugalization at high speed the supernatant fluid was again poured off, and the calcium bile pigment compound remained as a yellowish precipitate. The precipitate was washed free from other coloring matter and collected again by centrifugalization. Finally the precipitate dissolved in a hot solution of 5 per cent hydrochloric acid in 95 per cent alcohol gave the characteristic blue green color when bile pigments were present.

The method used to determine the amounts of bile pigments quantitatively will be published in the near future. This method in abstract consists of reading the blue green color of the acid alcohol extract in a fixed dilution against a permanent wedge of similar color which has been standardized against chemically pure bilirubin solutions of known amounts. The common sulphonephthalein colorimeter may be used to advantage.

Pleural Experiments.

Bile Pigments Formed from Hemoglobin in Periods of 8 to 66 Hours.

Dog 15-11.—8 and 24 hours. (Table I.) Short haired mongrel, male; weight 14 pounds.
Mar. 17. Dog is normal. 9.30 a.m. 20 cc. of concentrated urine are negative for bile pigments. 10 a.m. 20 cc. of washed, laked red blood cells are introduced into the right pleural cavity with 500 cc. of sterile 0.6 per cent salt solution. 11.10 a.m. No respiratory embarrassment. 2 p.m. 20 cc. of urine are negative for bile pigments and hemoglobin. 6 p.m. 100 cc. of dark red fluid removed from right pleural cavity are negative for bile pigments. 20 cc. of urine are negative for bile pigments and hemoglobin.

Mar. 18, 9.35 a.m. Dog is very active. Rectal temperature 39.1° C. 20 cc. of urine negative for bile pigments and hemoglobin. 10 a.m. 158 cc. of dark red fluid recovered from right pleural cavity are positive for bile pigments. 11.20 a.m. Right pleural cavity irrigated with 500 cc. of 0.6 per cent salt solution. Appetite is fairly good.

Mar. 19, 9 a.m. Dog is listless. Rectal temperature 40.2° C. Respiratory movements over right thorax are impaired.

Mar. 20, 10 a.m. Rectal temperature 39.1° C. Dog is very active and eats well. Respiration normal.

Dog 15-34.—8 hours. (Table I.) Mongrel setter, male; weight 55 pounds.

May 12. Dog is active and vigorous. 8.30 a.m. 20 cc. of urine are negative for bile pigments. 8.45 a.m. 500 cc. of water are introduced into the stomach through a stomach tube. 9.30 a.m. 30 cc. of washed, laked red blood cells are introduced into the right pleural cavity with 1,500 cc. of sterile 0.6 per cent salt solution. 11 a.m. 20 cc. of urine are negative for bile pigments and hemoglobin. 5 p.m. 20 cc. of urine are negative for bile pigments and hemoglobin. 5.30 p.m. 1,175 cc. of dark red, turbid fluid recovered from right pleural cavity. Chemical test is positive (suspicious) for bile pigments. 5.50 p.m. Right pleural cavity is irrigated with 500 cc. of 0.6 per cent salt solution. 6 p.m. Drinks some milk.

May 13, 9.30 a.m. Dog is listless and refuses to eat. Rectal temperature 102.3° F. Respiratory movements over right thorax are impaired, and a friction rub is palpable.

May 14, 10 p.m. Very active. Rectal temperature 101.8° F. Respiratory movements are normal.

Dog 15-39.—17 and 66 hours. (Table I.) Black mongrel, female; weight 32 pounds.

May 10. Dog is in excellent condition. 9.30 a.m. 20 cc. of concentrated urine are negative for bile pigments. 11.15 a.m. 30 cc. of washed, laked red blood cells suspended in 1,100 cc. of sterile 0.6 per cent salt solution are introduced into the right pleural cavity. 5.20 p.m. 930 cc. of dark red fluid, recovered from the right pleural cavity, give a positive (suspicious) test for bile pigments. 20 cc. of urine are negative for bile pigments and hemoglobin. 5.30 p.m. Right pleural cavity is irrigated with 500 cc. of 0.6 per cent salt solution. 6 p.m. Respiratory movements over right thorax are somewhat delayed.

May 11. Dog is active and vigorous. Rectal temperature 102.6° F. Respiratory movements are normal.
May 17. Dog is in excellent condition. Weight 32 pounds. 3.30 p.m. 20 cc. of urine are negative for bile pigments. 5 p.m. 30 cc. of washed, laked red blood cells are introduced into the left pleural cavity with 1,000 cc. of 0.6 per cent salt solution. 6.10 p.m. 20 cc. of urine are negative for bile pigments and hemoglobin. Respiratory movements are somewhat delayed over left thorax.

May 18, 9.30 a.m. Rectal temperature 103.2° F. Respiratory movements are markedly delayed over left thorax. 20 cc. of urine are negative for bile pigments and hemoglobin. 10 a.m. 390 cc. of dark red fluid recovered from the left pleural cavity contain 0.14 mg. of bile pigment. 5.30 p.m. Animal is quite active.

May 19. Dog is not very lively. 9 a.m. Rectal temperature 102.8° F.

May 25. Weight 32 pounds. Dog is active and vigorous. Rectal temperature 102.2° F. 4.30 p.m. 20 cc. of urine are negative for bile pigments. 4.45 p.m. 500 cc. of water are introduced into the stomach through a stomach tube. 4.50 p.m. The right pleural cavity is irrigated with 500 cc. of sterile 0.6 per cent salt solution. The fluid recovered is water-clear; bile pigment test negative. 6.10 p.m. 10 gm. of crystalline dog hemoglobin dissolved in 1,100 cc. of 0.6 per cent salt solution are introduced into the right pleural cavity. 6 p.m. Respiratory distress is not marked.

May 26. Dog is quite active and eats well. The movements of the right thorax are somewhat impaired. Rectal temperature 101.8° F. 2.10 p.m. 20 cc. of urine are negative for bile pigments and hemoglobin.

May 27. Rectal temperature 102.2° F. Movements of the right thorax are markedly impaired. Refuses food. 12.35 p.m. 20 cc. of urine are faintly positive for bile pigments.

May 28. Rectal temperature 102.8° F. 10.30 p.m. 20 cc. of urine are faintly positive for bile pigments. 11 a.m. 510 cc. of dark red turbid fluid recovered from the right pleural cavity contain 5.40 mg. of bile pigments. 11.20 a.m. Right pleural cavity is irrigated with 500 cc. of sterile 0.6 per cent salt solution.

May 29. Dog is normal.

Dog 15-52.—24 hours. (Table I.) Bull terrier mongrel, male; weight 41.5 pounds.

June 16. Dog is normal. 10 p.m. 20 cc. of urine are negative for bile pigments. 11.35 a.m. 9 gm. of crystalline dog hemoglobin dissolved in 1,000 cc. of 0.6 per cent salt solution are injected into the right pleural cavity. 12 m. Respiratory distress is not marked. 6 p.m. Animal drinks some milk.

June 17. Dog is quite active. Respiratory movements over right thorax are impaired. Rectal temperature 102.8° F. 10 a.m. 20 cc. of urine are negative for bile pigments. 11.30 a.m. 575 cc. of dark red, turbid fluid recovered from the right pleural cavity contain 0.18 mg. of bile pigment. 5 p.m. Respiratory movements are quite normal.

June 18. Dog is very active. Rectal temperature 102.4° F.
## ICTERUS

### TABLE I.

**Hemoglobin Changed to Bile Pigment in Pleural Cavity.**

<table>
<thead>
<tr>
<th></th>
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<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>15-11</td>
<td></td>
<td>lbs.</td>
<td>hrs.</td>
<td>mg.</td>
<td>cc.</td>
<td>cc.</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mar. 17</td>
<td>14.0</td>
<td>+</td>
<td>24</td>
<td>602</td>
<td>100</td>
<td>0</td>
<td>0</td>
<td>Fresh dog hb.</td>
</tr>
<tr>
<td>15-11*</td>
<td></td>
<td>lbs.</td>
<td>hrs.</td>
<td>mg.</td>
<td>cc.</td>
<td>cc.</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mar. 17</td>
<td>14.0</td>
<td>+</td>
<td>8</td>
<td>602</td>
<td>158</td>
<td>0</td>
<td>0</td>
<td>&quot; &quot; &quot;</td>
</tr>
<tr>
<td>15-34</td>
<td>55.0</td>
<td>+</td>
<td>8 (?)+</td>
<td>1,580</td>
<td>1,175</td>
<td>0</td>
<td>0</td>
<td>&quot; &quot; &quot;</td>
</tr>
<tr>
<td>15-39</td>
<td>32.0</td>
<td>+</td>
<td>7 (?)+</td>
<td>1,160</td>
<td>930</td>
<td>0</td>
<td>0</td>
<td>&quot; &quot; &quot;</td>
</tr>
<tr>
<td>15-40</td>
<td>22.0</td>
<td>+</td>
<td>6 (?)</td>
<td>700</td>
<td>555</td>
<td>0</td>
<td>0</td>
<td>&quot; &quot; &quot;</td>
</tr>
<tr>
<td>15-41</td>
<td>36.0</td>
<td>+</td>
<td>8 (?)+</td>
<td>1,480</td>
<td>1,230</td>
<td>0</td>
<td>0</td>
<td>&quot; &quot; &quot;</td>
</tr>
<tr>
<td>15-39</td>
<td>32.0</td>
<td>Left.</td>
<td>17 ++</td>
<td>0.14 1,105</td>
<td>0.99 805</td>
<td>265</td>
<td>0</td>
<td>&quot; &quot; &quot;</td>
</tr>
<tr>
<td>15-40</td>
<td>21.5</td>
<td>Left.</td>
<td>18 ++</td>
<td>0.09 805</td>
<td>0.09 805</td>
<td>265</td>
<td>0</td>
<td>&quot; &quot; &quot;</td>
</tr>
<tr>
<td>June 16</td>
<td>41.5</td>
<td>Right.</td>
<td>24 +++++</td>
<td>0.18 1,000</td>
<td>0.18 1,000</td>
<td>575</td>
<td>0</td>
<td>Crystalline dog hb., 10 gm.</td>
</tr>
<tr>
<td>15-54</td>
<td>36.5</td>
<td>Right.</td>
<td>24 +</td>
<td>800</td>
<td>395</td>
<td>0</td>
<td>0</td>
<td>&quot; &quot; &quot; 7 &quot;</td>
</tr>
<tr>
<td>June 16</td>
<td>29.5</td>
<td>Right.</td>
<td>25 +</td>
<td>800</td>
<td>408</td>
<td>0</td>
<td>0</td>
<td>&quot; &quot; &quot; 7 &quot;</td>
</tr>
<tr>
<td>15-56</td>
<td>39.0</td>
<td>Left.</td>
<td>25 +</td>
<td>900</td>
<td>318</td>
<td>0</td>
<td>0</td>
<td>&quot; &quot; &quot; 7 &quot;</td>
</tr>
<tr>
<td>June 16</td>
<td>36.5</td>
<td>Left.</td>
<td>43 ++++</td>
<td>0.15 805</td>
<td>0.15 805</td>
<td>254</td>
<td>0</td>
<td>(faint) Fresh &quot; &quot;</td>
</tr>
<tr>
<td>15-43</td>
<td></td>
<td>lbs.</td>
<td>hrs.</td>
<td>mg.</td>
<td>cc.</td>
<td>cc.</td>
<td></td>
<td></td>
</tr>
<tr>
<td>May 20</td>
<td>34.0</td>
<td>Right.</td>
<td>44 +++++</td>
<td>0.36 1,100</td>
<td>1,100</td>
<td>808</td>
<td>0</td>
<td>Crystalline &quot; &quot; 4 &quot;</td>
</tr>
<tr>
<td>15-40</td>
<td></td>
<td>lbs.</td>
<td>hrs.</td>
<td>mg.</td>
<td>cc.</td>
<td>cc.</td>
<td></td>
<td></td>
</tr>
<tr>
<td>May 25</td>
<td>22.0</td>
<td>+</td>
<td>65 +++++</td>
<td>3.60 940</td>
<td>940</td>
<td>430</td>
<td>0</td>
<td>+ (faint) Fresh &quot; &quot;</td>
</tr>
<tr>
<td>15-30*</td>
<td></td>
<td>lbs.</td>
<td>hrs.</td>
<td>mg.</td>
<td>cc.</td>
<td>cc.</td>
<td></td>
<td></td>
</tr>
<tr>
<td>May 25</td>
<td>32.0</td>
<td>+</td>
<td>66 +++++</td>
<td>5.40 1,100</td>
<td>1,100</td>
<td>510</td>
<td>0</td>
<td>+ (faint) Crystalline &quot; &quot; 10 &quot;</td>
</tr>
</tbody>
</table>

*See history for details.
Dog 15-43.—43 hours. (Table I.) Mongrel spaniel, male; weight 34 pounds.
May 20. Dog is in excellent condition. Rectal temperature 102° F. 12.10 p.m. 20 cc. of urine are negative for bile pigments. 12.15 p.m. 500 cc. of water are introduced into the stomach through a stomach tube. 3.10 p.m. 4 gm. of crystalline dog hemoglobin dissolved in 1,100 cc. of sterile 0.6 per cent salt solution are introduced into the right pleural cavity. 5.30 p.m. 20 cc. of urine are negative for bile pigments and hemoglobin.
May 21. Dog is active and eats well. Respiratory movements over the right thorax are impaired. Rectal temperature 102.2° F.
May 22. Rectal temperature 103.1° F. 10.30 a.m. 20 cc. of urine are negative for bile pigments and hemoglobin. 11 a.m. 808 cc. of dark red, turbid fluid recovered from the right pleural cavity contain 0.36 rag. of bile pigments.
May 23. Rectal temperature 101.8° F. Dog is normal.

The preceding experiments are conclusive proof that mesothelium can rapidly transform hemoglobin into bile pigment. The lining cells of the pleural cavity like other body cells (probably endothelium) can form bile pigments out of hemoglobin, either freshly laked or crystallized. This solution of hemoglobin is not in very intimate contact, not in circulatory contact, as is hemoglobin in contact with the endothelium of the capillaries, yet the transformation takes place promptly in a few hours. There is experimental evidence of this bile pigment transformation in the pleura within eight hours, but a very definite amount is formed within eighteen to twenty-four hours, an amount often sufficient for accurate determination.

Dog 15-20.—8 hours. (Table II.) Mongrel poodle, male; weight 19.5 pounds.
Mar. 29. Dog is normal, 10 a.m. 20 cc. of concentrated urine are negative for bile pigments. 10.30 a.m. 35 cc. of washed, laked red blood cells are introduced into the peritoneal cavity with 800 cc. of 0.6 per cent salt solution. 12.30 a.m. 20 cc. of urine are negative for bile pigments and hemoglobin. 2 p.m. Rectal temperature 101.3° F. 5.30 p.m. 20 cc. of urine are negative for bile pigments and hemoglobin. 6.30 p.m. 300 cc. of dark red turbid fluid recovered from the peritoneal cavity give a questionably positive test for bile pigments. 6.45 p.m. Peritoneal cavity is irrigated with 2,000 cc. of 0.6 per cent salt solution. Urine gives positive (suspicious) test for bile pigments.
Mar. 30. Dog is very lively and eats well. Rectal temperature 101.7° F.

Dog 15-43.—28 hours. (Table II.) Mongrel spaniel, male; weight 33.5 pounds.
June 17. Dog is in excellent condition. 9.45 a.m. 20 cc. of urine are negative for bile pigments. 11.15 a.m. 7 gm. of crystalline dog hemoglobin dissolved in 2,800 cc. of 0.6 per cent salt solution, introduced into the peritoneal cavity.
12 m. Dog shows no embarrassment. 5.30 p.m. Animal listless and refuses to eat. Considerable rigidity of abdomen.

June 18. Rectal temperature 103.8°F. Animal is listless, will not eat, and lies quietly in cage. Abdominal muscles are very tense. 2 p.m. 20 cc. of urine are positive for bile pigments. 3.20 p.m. 755 cc. of dark red turbid fluid recovered from the peritoneal cavity give a positive test for bile pigments. 3.35 p.m. Peritoneal cavity irrigated with 3,000 cc. of sterile 0.6 per cent salt solution. 5 p.m. Rectal temperature 104.2°F.

June 19. 9 a.m. Animal is found dead. Autopsy showed a diffuse fibrinous peritonitis. Other organs are negative.

Dog 15-55.—48 hours. (Table II.) Mongrel hound, male; weight 40.5 pounds.

June 16. Dog is in excellent condition. 2 p.m. 20 cc. of urine are negative for bile pigments. 2.40 p.m. 7 gm. of crystalline dog hemoglobin dissolved in 3,000 cc. of 0.6 per cent salt solution introduced into the peritoneal cavity. 6 p.m. Dog is quite active. 20 cc. of urine are negative for bile pigments and hemoglobin.

June 17. Rectal temperature 102.2°F. 10.10 a.m. 20 cc. of urine are positive for bile pigments. Hemoglobin negative.

June 18. Dog is very lively and eats well. 9.30 a.m. Rectal temperature 103.6°F. 1.30 p.m. 20 cc. of urine are negative for bile pigments and hemoglobin. 2.30 p.m. 310 cc. of dark red, turbid fluid recovered from peritoneal cavity contain 0.27 mg. of bile pigment. 2.50 p.m. Peritoneal cavity irrigated with 2,000 cc. of 0.6 per cent salt solution. 5.30 p.m. Dog is very active.

June 30. Dog is normal.

**TABLE II.**

**Hemoglobin Changed to Bile Pigment in Peritoneal Cavity.**

<table>
<thead>
<tr>
<th>Dog No.</th>
<th>Date</th>
<th>Weight</th>
<th>Time in peritoneal cavity</th>
<th>Bile pigment tests</th>
<th>Bile pigment introduced</th>
<th>Fluid recovered</th>
<th>Bile in urine</th>
<th>Remarks</th>
</tr>
</thead>
<tbody>
<tr>
<td>1915</td>
<td></td>
<td>lbs.</td>
<td>hrs.</td>
<td>-</td>
<td>-</td>
<td>0</td>
<td>(+)</td>
<td>Fresh dog hb.</td>
</tr>
<tr>
<td>15-20</td>
<td>May 20</td>
<td>56.0</td>
<td>44</td>
<td>++++++</td>
<td>0.25</td>
<td>4,000</td>
<td>1,360</td>
<td>&quot;    &quot;  &quot;  7  &quot;</td>
</tr>
<tr>
<td>15-41</td>
<td>June 17</td>
<td>36.5</td>
<td>48</td>
<td>++++++</td>
<td>0.18</td>
<td>3,000</td>
<td>310</td>
<td>&quot;    &quot;  &quot;  7  &quot;</td>
</tr>
<tr>
<td>15-57</td>
<td>June 16</td>
<td>24.5</td>
<td>48</td>
<td>++++++</td>
<td>0.32</td>
<td>2,500</td>
<td>255</td>
<td>&quot;    &quot;  &quot;  7  &quot;</td>
</tr>
<tr>
<td>15-55</td>
<td>June 16</td>
<td>40.5</td>
<td>46</td>
<td>++++++</td>
<td>0.27</td>
<td>3,000</td>
<td>310</td>
<td>&quot;    &quot;  &quot;  7  &quot;</td>
</tr>
</tbody>
</table>
The preceding experiments and Table II show that the peritoneal cavity can transform hemoglobin into bile pigment with the same promptness as the pleural cavity. The two cavities are lined by a similar mesothelium, so there need be no surprise when the reactions are found to be similar. The difference in absorption from the two cavities is obvious. The pleural cavity gives slow absorption, most of the fluid introduced is recovered, and little if any of the formed bile pigment appears in the urine. The peritoneal cavity gives a pretty rapid absorption, most of the fluid introduced is taken up, and the bile pigments appear in the urine as a result.

DISCUSSION.

A difference in absorption from the pleural and peritoneal cavities has been noted and it comes out clearly in Tables I and II. The relatively rapid absorption from the peritoneal cavity causes the escape of bile pigments in the urine. Even in the pleural experiments, however, there is considerable absorption which indicates that in both pleural and peritoneal cavities there is an outflow of fluid. Whether there is interchange between the introduced fluid and the body fluids is uncertain, but much interchange is unlikely. In these experiments the dogs were all normal and their urine free from bile pigment at the beginning of the experiments, which means that the serum was negative to bile pigments by the test employed. That the blood pigments could have been absorbed into the blood, changed to bile pigments in association with the liver, and again diffused into the pleural fluid, is inconceivable. The observations in the pleural experiments (Table I) show that the urine was free from hemoglobin and bile pigments during the first forty-eight hours.

A theoretical objection could be raised to the experiments with the hemoglobin solutions in the peritoneal cavity. It could be argued that this solution comes in contact with the liver and could be modified in this manner. The solution does come in contact with the serous covering of the liver, but not with the hepatic epithelium. Diffusion through the serous epithelium of the liver is conceivable, but most improbable, and the pleural experiments are not open to such an objection.
The use of crystalline hemoglobin seems to cause more irritation of the serous surfaces than does a fresh solution of hemoglobin obtained from freshly laked red corpuscles. When this irritant action lasts over a period of three days there is undoubtedly some new formation of capillaries and proliferation of mesothelium, as well as an escape of various wandering cells. This reaction brings other extrahepatic factors into the equation and apparently accelerates the formation of bile pigment (Table I).

All this evidence indicates that the function of changing hemoglobin to bile pigment is not limited to any single cell or even to two types of cell. It may well be a function of endothelium and mesothelium as well as of hepatic epithelium. Perhaps wandering cells have this property of transforming blood to bile pigment. Many of these wandering cells probably have their origin from endothelium, and may they not retain this functional capacity? It is quite possible that other epithelium besides liver epithelium may be able to effect this transformation. More experiments are being carried out with this point in view. If we go a step further it may be suggested that living protoplasm in general can change hemoglobin to bile pigments.

Still other questions may be raised. Can tissue juices or ferments bring about this transformation of hemoglobin to bile pigment? We have performed many experiments with negative results, but have not yet given up the attempt although success seems unlikely.

CONCLUSIONS.

It is known that hemoglobin can be rapidly changed to bile pigment in a circulation confined to the head, neck, and thorax. This excludes direct liver participation (1).

These experiments show that hemoglobin can be changed to bile pigment within the pleural or peritoneal cavities.

This transformation can usually be detected after eight hours, and the amount can often be estimated quantitatively after an interval of twenty-four hours.

Such experiments demonstrate the importance which may attach to extrahepatic bile pigment formation. That bile pigments can be formed without direct liver activity is established beyond doubt.
It is highly probable that endothelium as well as mesothelium (serous cavities) can transform hemoglobin into bile pigment. It is possible that this property may reside in cell protoplasm in general.

BIBLIOGRAPHY.